

of stimulus being placed in round numbers 2 per cent. below the arbitrary zero, that of physiological response in round numbers 20 per cent. below the arbitrary point of origin (0') of the empirical curve.

At the same time this 20 per cent. estimate is little better than a guess. I am of opinion that the time for an absolute scale is not yet, and that an empirical generalisation in close touch with observation and experiment, such as that on which my own curve is based, is more likely to be helpful as a guide to further investigation than a wider law involving assumptions the validity of which is doubtful.

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"The Yellow Colouring Matters accompanying Chlorophyll and their Spectroscopic Relations. Part II." By C. A. SCHUNCK. Communicated by Dr. E. SCHUNCK, F.R.S. Received June 5, —Read June 20, 1901.

[PLATES 5, 6.]

In the former investigation\* the yellow colouring matters, generally known as the xanthophyll group, which accompany chlorophyll in the healthy green leaves, and which are extracted along with it by means of alcohol, were separated from the chlorophyll by treating the alcoholic extracts with an excess of animal charcoal in the cold, by which means the chlorophyll is absorbed by the charcoal, leaving the yellow colouring matters in the alcohol. On investigating this crude yellow solution it became evident that more than one colouring matter was present, and I now give the results of the experiments I have made in the endeavour to further isolate the constituents of this group by means of carbon bisulphide, which method was adopted by Sorby† in his investigation of the different colouring matters present in plants. The crude alcoholic extracts of the accompanying yellow colouring matters, which I will for the future term the xanthophylls, can be obtained either by the above method or by boiling the chlorophyll extracts for three or four hours with caustic potash or soda (10 grammes to 1 litre of solution), allowing to stand, and shaking up with ether, which takes up the xanthophylls unaltered, whereas the chlorophyll is changed to an alkali compound of alka-chlorophyll, which is insoluble in ether, but soluble in water; the ethereal solution is then evaporated, and the residue dissolved in alcohol. From either method of preparation the same results are obtained.

These crude yellow alcoholic solutions of the xanthophylls show, as a general rule, four distinctive absorption bands in the violet and ultra-violet situated between the lines F' and L (Plate 5, C, 2), any indication of

\* 'Roy. Soc. Proc.,' vol. 65, p. 177.

† 'Roy. Soc. Proc.,' vol. 21, p. 456.

a band in the red being due to a trace of chlorophyll that has not been removed, but in some instances depending upon the particular plant experimented with, and the same plant at different seasons, The fourth and most refrangible band is extremely faint, if not absent (Plate 6, F, 1). This variation of the spectrum I failed to observe in my former experiments, and its significance will be apparent later on. As pointed out in my former investigation,\* in some instances only the first two or three bands are visible, the rest of the violet and ultra-violet being obscured by a yellow colouring matter producing general absorption, but no bands, which, according to Sorby, belongs to his Lichnoxanthine group, and corresponds to the so-called xanthophyll of Tschireh. In such cases a separation can be effected by agitating with ether, and the addition of water, the colouring matter causing the obscuration remaining in the watery alcoholic solution. In every case the extent of the ultra-violet visible varies, depending, as before, upon the particular plant experimented upon and the same plant at different seasons.

Most of the present experiments were made with alcoholic extracts obtained from *Ficus Carica* and *Ficus Repens*, both of which give a very excellent chlorophyll spectrum, pointing to the presence of very little acid in the juices of the leaf, and, as the presence of acid affects the xanthophylls, it is of importance to prevent complications to experiment with a plant that is more or less free from acid in its juices, a delicate indication of which is the condition of the chlorophyll spectrum of the alcoholic extract, whether normal or not, for the least trace of acid will cause the fourth chlorophyll band to become pronounced instead of appearing very faint. The observations of the absorption spectra were effected as before by means of photography, quartz lenses and an Iceland spar prism being used, and the source of light was a Welsbach incandescent gas mantle of 60-candle power.

The method of procedure was to agitate the crude alcoholic solution of the xanthophylls from which the chlorophyll had been removed by one of the above means with successive equal volumes of  $\text{CS}_2$  until no more colouring matter was taken up by the  $\text{CS}_2$ , each volume of  $\text{CS}_2$  being equal to about half the volume of the crude solution experimented upon. By this means we have the colouring matters capable of being taken up by  $\text{CS}_2$  divided into several  $\text{CS}_2$  portions or fractions (which varied from six to twelve according to the concentration of the crude solution) according to their relative solubility, leaving in the alcohol those colouring matters which are more soluble in it than in  $\text{CS}_2$ .

On examining first the alcoholic portion from which the dissolved  $\text{CS}_2$  had been evaporated by gentle heat, it is found to be a paler yellow than the crude solution and to give four absorption bands in

\* 'Roy. Soc. Proc.' vol. 65, p. 181.

the violet and ultra-violet, the first two and least refrangible of which are slightly but distinctly shifted towards the violet compared to the first two bands in the crude solution, while the other two occupy approximately the same positions (Plate 5, A, 3); but it is only in a few instances they are plainly visible on the photographic plate, usually they are more or less obscured, only the first band being distinct and well defined (Plate 6, F, 2). The obscuration is no doubt due to the yellow colouring matter before mentioned, the greater quantity of which remains in the alcohol after the  $\text{CS}_2$  fractionation, being more soluble in the former than in the latter. Its presence in a considerable quantity tends further to obscure the bands, and it can then be detected at once by the alcoholic solution after fractionation, being more of a straw colour than the usual pale yellow. This spectrum is not stable, for, after standing a few days the least refrangible band fades and finally disappears, and, after a further lapse of time, the other three bands, more especially the third and fourth, became intensified and well defined, the rest of the ultra-violet being obscured (Plate 6, F, 4); but in some cases when there is very little obscuration present, an additional band is discernible in the ultra-violet (Plate 5, A, 4). This change, however, only takes place, as a rule, after fractionation, as the crude solution can be kept a considerable time without any change taking place, pointing to the capability of one colouring matter in protecting a less stable one in a mixture. The same change, however, can be effected at once by adding a very small quantity of  $\text{HCl}$  to the alcoholic portion, when the colour of the solution immediately becomes a paler yellow, but in a few hours all the bands disappear and the solution becomes a peacock-blue colour which, in a day or two, likewise fades leaving the solution finally colourless. This blue coloration is a characteristic of the colouring matter left in the alcohol after fractionation. By agitating the alcoholic portion with ether and adding water till a separation takes place, the ether takes up the greater quantity of the colouring matter, and, on spontaneous evaporation, an amorphous lemon-yellow substance is deposited which also gives this same spectrum. The last of the  $\text{CS}_2$  fractions when taken into alcohol in some cases likewise give this spectrum.

The question whether the normal spectrum of the alcoholic portion represents a single colouring matter I have been unable to decide definitely by spectroscopic means, but I think the above facts tend to prove that on standing or by the action of  $\text{HCl}$  a definite colouring matter is formed therefrom giving the above changed spectrum, and from it, by the further action of acid, a blue colouring matter is produced. I also think the experiments tend to show that this colouring matter does not pre-exist in the leaf, but is formed subsequently, either spontaneously or by the action of the acid juices during or after the process of extraction, which is supported by the fact that from extracts

of such leaves as ivy and Virginia creeper that contain much acid in their juices, as evidenced by the condition of their chlorophyll spectrum, the alcoholic portion exhibits this changed spectrum, but if means be taken during extraction to neutralise the acid the normal spectrum is obtained.

Sorby considers\* that the alcoholic portion, in addition to his lichnoxanthine, contains two colouring matters which he terms xanthophyll and yellow xanthophyll, and that the action of acid on the *latter* produces the colouring matter giving the above changed spectrum and afterwards the blue coloration.

The CS<sub>2</sub> fractions were evaporated at a gentle heat to dryness, and taken up with alcohol and examined successively. In the first one or two fractions the ultra-violet is visible to a considerable extent, the spectrum consisting of three pronounced well-defined bands, which are slightly shifted, more especially the first towards the red end as compared to the first three bands in the crude solution, the fourth band being absent. The subsequent fractions one by one transmit less and less of the ultra-violet, the three bands are gradually shifted little by little towards the violet in succeeding fractions, the first band gradually becomes fainter, while a fourth band more refrangible than the other three, makes its appearance and becomes intenser as we pass from fraction to fraction, and it will be found that one of the latter fractions corresponds in its spectrum to that of the crude solution (Plate 5, B, 1-5). Lastly the final fractions as a rule exhibit the spectrum produced by acid on the alcoholic portion, the colouring matter to which it is due appearing to be more soluble in CS<sub>2</sub> than in alcohol. The greater part of the colouring matter is found in the first two or three fractions, which are coloured a rich yellow, the succeeding fractions becoming paler and paler until the final fractions are almost colourless, and in order to exhibit their spectra have to be greatly concentrated.

The interpretation of this series of spectra is, I believe, that the crude solution is a mixture of chrysophyll and the colouring matters or matter remaining in the alcohol after fractionation, together with the colouring matter formed from the latter by the action of acid. For on comparing the spectrum of the first fractions with that of chrysophyll they are identical, save that the bands in the former are very slightly shifted towards the violet (Plate 5, D, 1-2); these first fractions also transmit the ultra-violet to a considerably greater extent than does the crude solution, which, together with the three pronounced bands, is a characteristic of chrysophyll; and further, if we mix chrysophyll and the colouring matter remaining in the alcohol after fractionation together, in proper proportions, the spectrum obtained is identical with that of the crude solution (Plate 6, E, 2 and 3). Likewise, I believe,

\* 'Roy. Soc. Proc.,' vol. 21, p. 459.

the various fractions contain these same colouring matters in different proportions, depending upon their relative solubility in  $\text{CS}_2$  and alcohol, which is borne out by the slight differences in their spectra, as we pass in rotation from the first to the last fraction. Chrysophyll, as is well known, is always found deposited in the form of sparkling red crystals from the crude chlorophyll extracts when concentrated sufficiently on standing, but in one case only have I been able to obtain the crystals from a crude solution of the xanthophylls after removing the chlorophyll, though I have made many attempts. The failure in this respect may perhaps be accounted for by the very great difficulty there always is in even re-crystallising this substance.

That the bands are not quite in identical positions is admissible, for one cannot obtain a complete separation by a method that depends upon the relative solubility of its constituents in two solvents, so that we should expect to find in the first fractions a little of the other colouring matters (which tend to produce the shifting of the bands), together with the greater portion of the chrysophyll.

From the above results it is, I think, evident that chrysophyll pre-exists, and is not formed spontaneously from one of the colouring matters of the leaf as has been held by some observers, and that it is one if not the chief constituent of the xanthophyll group of yellow colouring matters, accompanying chlorophyll in the healthy green leaf. Chrysophyll evidently corresponds to the orange xanthophyll of Sorby,\* which he states is one of the most universally distributed of all vegetable colouring matters, occurring in greater or less quantity in all classes of plants, including fungi.

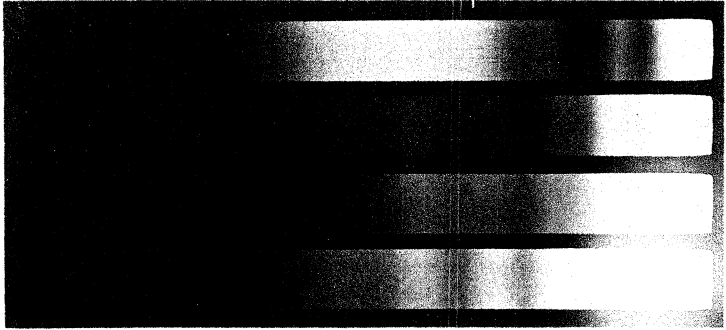
The action of acid upon the spectrum of chrysophyll, the first  $\text{CS}_2$  fractions, the alcoholic portion and the crude solution of the xanthophylls is instructive when compared together, and tends further to confirm the above view taken of the constitution of the crude solution. If a small quantity of  $\text{HCl}$  be added to each, the effect upon the chrysophyll spectrum and that of the first  $\text{CS}_2$  fractions is to cause the bands to fade and the solutions to become gradually colourless (Plate 6, G, 1 and 2). In the crude solution the effect is to cause the first band to become fainter and the fourth darker, even though it be extremely faint, as I have pointed out is the case in some crude solutions. The bands then after a short time fade, but the solution assumes a green colour before becoming colourless (Plate 6, G, 3 and 4).

Lastly, in the alcoholic portion, as before stated, the effect of the acid is to remove the first band, and to clear up the spectrum, the three remaining bands becoming intensified, especially the third and fourth. The bands then fade and the solution assumes a peacock-blue colour, which also after a short time fades, leaving the solution colourless (Plate 6, G, 5). Thus the effect of acid in causing the first band to fade

\* 'Roy. Soc. Proc.,' vol. 21, p. 457.

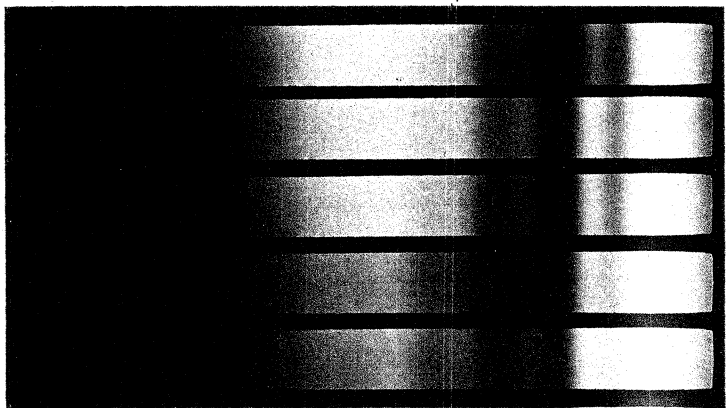
Q P O N M L K $\beta$  G F

A



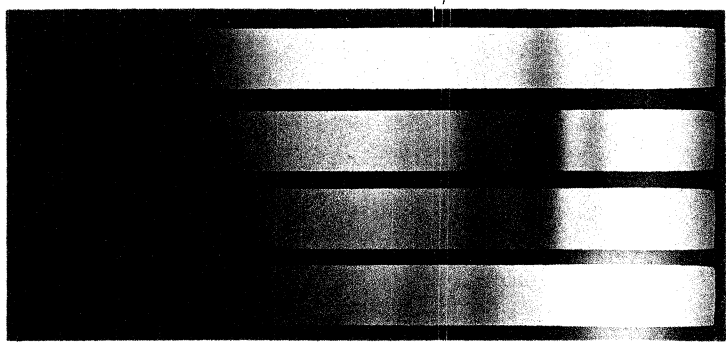
K $\beta$

B



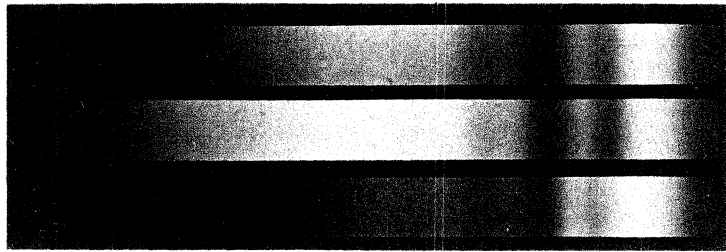
K $\beta$

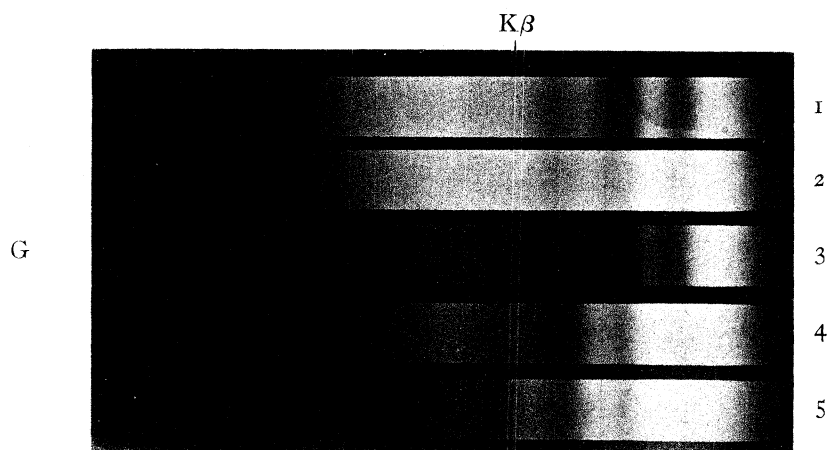
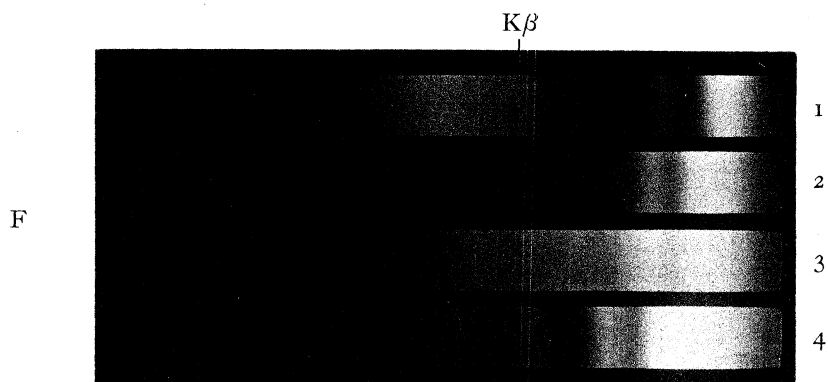
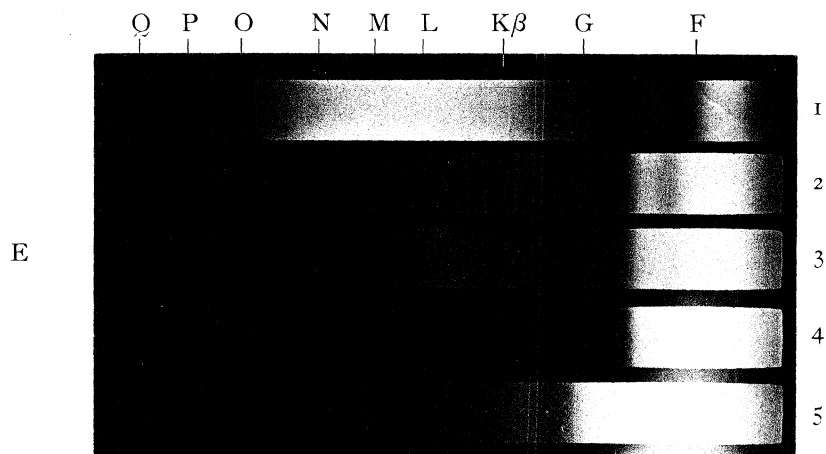
C



K $\beta$

D





and the fourth to intensify in the crude solution, together with its effect upon the alcoholic portion and chrysophyll, is in accordance with the view that it is a mixture of the two. I also think the action of acid upon the spectrum of the alcoholic portion explains the origin of the fourth band in the crude solution and its appearance in the later  $\text{CS}_2$  fractions, and conclude that it is due to the colouring matter giving the changed spectrum, and formed from the alcoholic portion, either spontaneously or by the action of the acid juices during or after extraction, and that its variability in intensity depends upon the amount of this colouring matter formed. If there be but little acid present, or if means be taken to neutralise it during extraction, then the band will appear, but faint, and in some cases perhaps absent. The green colour assumed by the crude solution is no doubt due to the formation of the peacock-blue colouring matter, which, mixed with the yellow chrysophyll, causes the solution to appear green.

From the above experiments I was evidently in the wrong in the former investigation in considering that the four-banded spectrum exhibited by the crude solution of the xanthophylls represented a single colouring matter, to which I restricted the name Xanthophyll, and think now the right interpretation is that this spectrum is due to a mixture of colouring matters, the chief constituent of which I have been led to believe from the above facts is Chrysophyll, the only member so far of the accompanying yellow colouring matters, I believe, that is obtainable in a crystalline form.

#### EXPLANATION OF PLATES.

(The solvent in every case is Alcohol.)

#### PLATE 5.

Xanthophylls obtained from an extract of *Ficus Repens* in the month of February :—

- A. (1) The first  $\text{CS}_2$  fraction.
- (2) The thirteenth and final fraction.
- (3) The alcoholic portion, showing in this experiment four distinct bands.
- (4) The above +  $\text{HCl}$ , in this experiment showing a distinct fourth band.
- B. Some of the  $\text{CS}_2$  fractions in alcohol :—
  - (1) The first; (2) the second; (3) the fifth; (4) the seventh; (5) the ninth.
- C. (1) The first  $\text{CS}_2$  fraction.
- (2) The crude solution of the xanthophylls.
- (3) A mixture of the first  $\text{CS}_2$  fraction and the alcoholic portion.
- (4) The alcoholic portion.
- D. Comparison of—
  - (1) The first  $\text{CS}_2$  fraction.
  - (2) Chrysophyll.
  - (3) The crude solution of the xanthophylls in which the fourth band in this instance is faint.



## PLATE 6.

E. Xanthophylls obtained from an extract of *Ficus Repens* in the month of May:—

- (1) Chrysophyll obtained from the crude solution of the xanthophylls.
- (2) Crude solution of the xanthophylls.
- (3) A mixture of chrysophyll and the alcoholic portion.
- (4 and 5) The alcoholic portion of different strengths, showing a slight obscuration.

F. Xanthophylls obtained from an extract of *Ficus Repens* in the month of December:—

- (1) Crude solution of the xanthophylls ; a case in which the fourth band is almost, if not, absent.
- (2 and 3) The alcoholic portion of different strengths. This is the usual appearance of this spectrum, showing the bands more or less obscured.
- (4) The alcoholic portion after standing a little time, the spectrum being the same as that produced immediately by the action of HCl.

G. The action of HCl on the xanthophylls :—

- (1) The first CS<sub>2</sub> fraction.
- (2) The first CS<sub>2</sub> fraction + HCl.
- (3) Crude solution of the xanthophylls.
- (4) Crude solution of the xanthophylls + HCl.
- (5) Alcoholic portion (F - 2) + HCl.

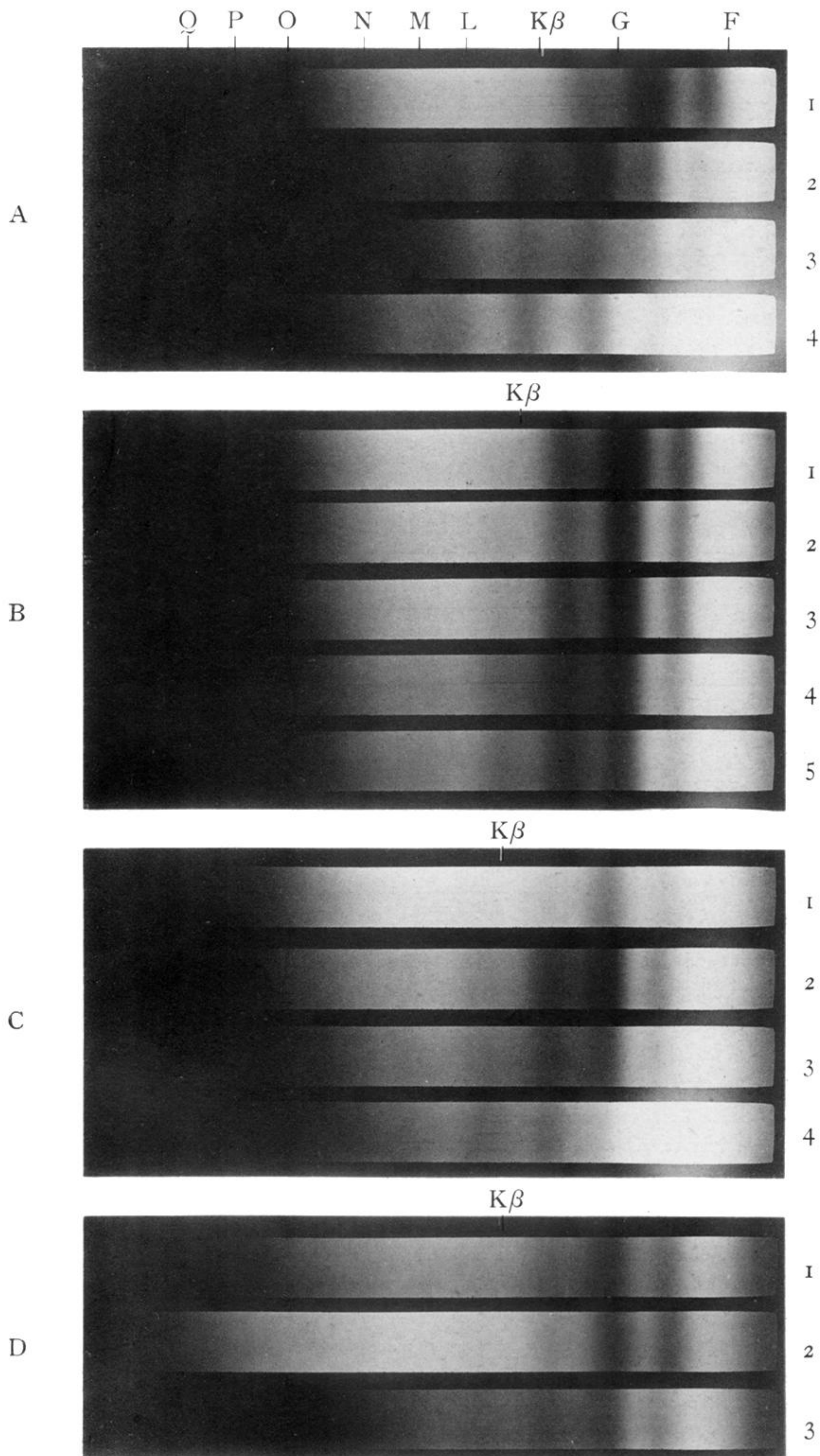
“On Skin Currents.—Part I. The Frog’s Skin.” By AUGUSTUS D. WALLER, M.D., F.R.S. Received May 29,—Read June 6, 1901.

The principal object of the following observations was to investigate in the case of skin an electrical reaction by which it is in general possible to determine whether an animal or vegetable tissue is alive or dead.\*

A side issue raised in connection with the general inquiry was whether or no the test is applicable to the human body ; this obviously led to a detailed study of skin effects upon man and upon animals.

In the case of the frog, previous observations on skin currents are numerous and conflicting ; but in so far as my present theme is concerned, the results have come out with the utmost regularity and quite clear of any suspicion of physical fallacy. In the case of man, the question has proved to be less simple, and although it is easy to distinguish between an assuredly living and an assuredly dead piece of skin, it is far from easy in doubtful cases to make sure that the skin is completely dead. The difficulty is caused by polarisation currents

\* ‘Roy. Soc. Proc.’ vol. 68, p. 79. References to previous papers are given there—p. 92.



### EXPLANATION OF PLATES.

(The solvent in every case is Alcohol.)

#### PLATE 5.

Xanthophylls obtained from an extract of *Ficus Repens* in the month of February :—

- A. (1) The first CS<sub>2</sub> fraction.  
 (2) The thirteenth and final fraction.  
 (3) The alcoholic portion, showing in this experiment four distinct bands.  
 (4) The above + HCl, in this experiment showing a distinct fourth band.
- B. Some of the CS<sub>2</sub> fractions in alcohol :—  
 (1) The first; (2) the second; (3) the fifth; (4) the seventh; (5) the ninth.
- C. (1) The first CS<sub>2</sub> fraction.  
 (2) The crude solution of the xanthophylls.  
 (3) A *mixture* of the first CS<sub>2</sub> fraction and the alcoholic portion.  
 (4) The alcoholic portion.
- D. Comparison of—  
 (1) The first CS<sub>2</sub> fraction.  
 (2) Chrysophyll.  
 (3) The crude solution of the xanthophylls in which the fourth band in this instance is faint.



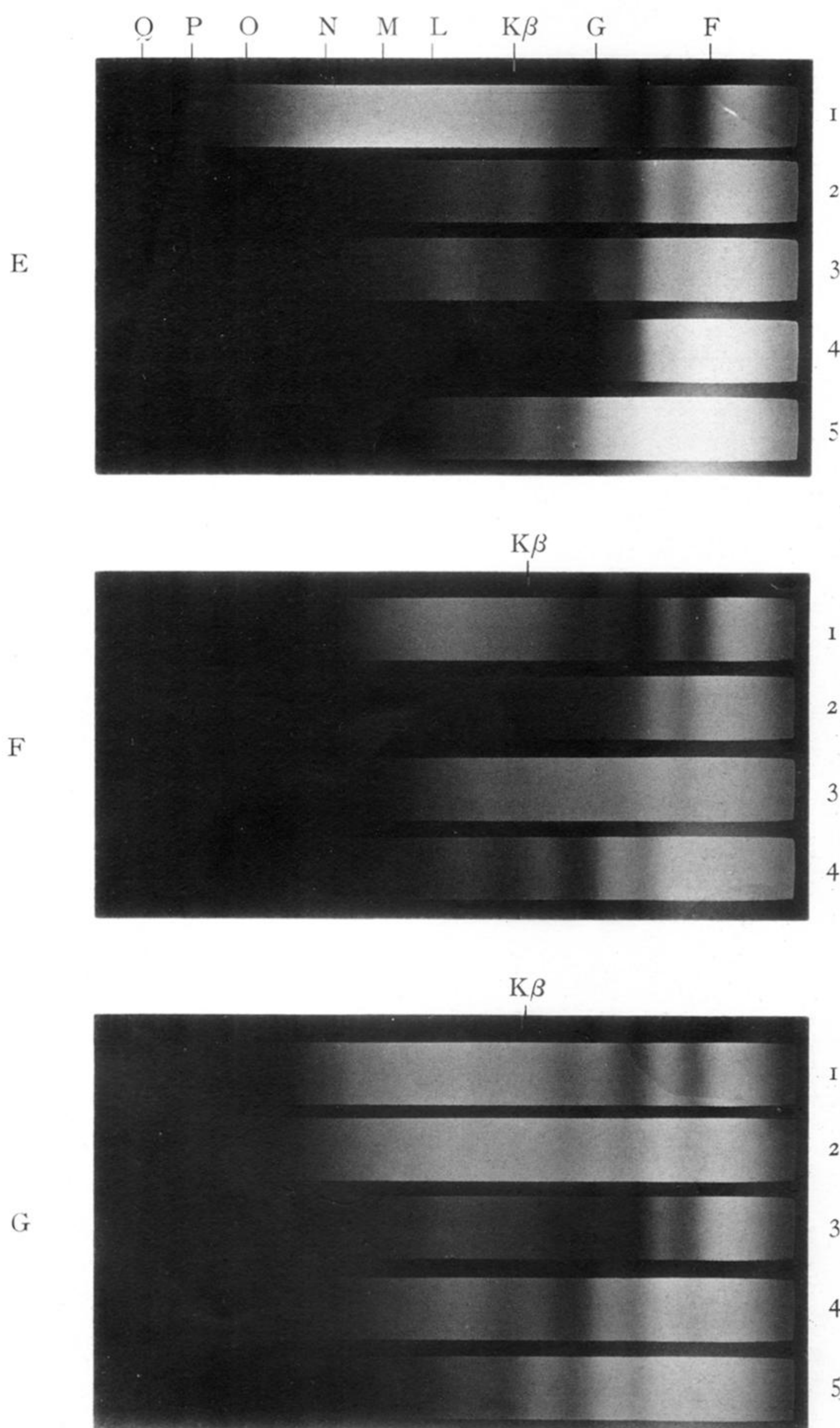


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- (1) Chrysophyll obtained from the crude solution of the xanthophylls.
- (2) Crude solution of the xanthophylls.
- (3) A mixture of chrysophyll and the alcoholic portion.
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F. Xanthophylls obtained from an extract of *Ficus Repens* in the month of December :—

- (1) Crude solution of the xanthophylls ; a case in which the fourth band is almost, if not, absent.
- (2 and 3) The alcoholic portion of different strengths. This is the usual appearance of this spectrum, showing the bands more or less obscured.
- (4) The alcoholic portion after standing a little time, the spectrum being the same as that produced immediately by the action of HCl.

G. The action of HCl on the xanthophylls :—

- (1) The first CS<sub>2</sub> fraction.
- (2) The first CS<sub>2</sub> fraction + HCl.
- (3) Crude solution of the xanthophylls.
- (4) Crude solution of the xanthophylls + HCl.
- (5) Alcoholic portion (F - 2) + HCl.